~~[Name of document]~~ ~~SPECIFICATION~~

METHODS FOR CONSTRUCTING A DNA LIBRARY AND A SUPPORT CARRYING A DNA LIBRARY IMMOBILIZED THEREON

~~TECHNICAL~~ FIELD OF THE INVENTION

[0001] ~~An industrial field of~~ tThe present invention relates to ~~a~~ molecular ~~biological technical~~ biology and ~~a~~ biochemical ~~technical such as a~~ gene technology, ~~a~~ protein technology, ~~a~~ and cell technology ~~and an immunology technology,~~. More particularly, the present invention relates to a method for ~~constructing~~ construction of a plurality of ~~an original support~~supports on which a ~~Deoxyribonucleic Acid (hereinafter, it is referred as "DNA")~~DNA library is immobilized ~~by utilizing~~ using a micro amount of DNA test material, a method for constructing ~~its replica~~ the supports, and ~~a support~~supports on which DNA is immobilized ~~with a DNA piece~~.

~~BACKGROUND OF THE INVENTION~~ **BACKGROUND OF THE INVENTION**

[0002] DNA is a very important test material. In ~~a~~ conventional ~~art~~ methods for working with~~, in the case of experimenting~~ DNA, DNA is amplified ~~by utilizing~~ using a

~~Polymerase Chain Reaction~~Polymerase Chain Reaction (hereinafter, ~~it is referred as "~~PCR~~"~~) and divided to small groups. ~~since DNA is very important test material.~~ The DNA test material is preserved at ~~a~~ remarkably low ~~temperature~~ temperatures in a freezer. ~~In a conventional art~~Conventionally, a DNA library is produced in a solution ~~condition~~ so that a replica of the DNA library ~~can not~~ cannot be produced. Accordingly, one must work ~~it has to treat a DNA library test material in a solution condition obtained from micro amount of tissue or cells~~ very carefully with a DNA library in solution obtained from tissue or cells in order to search and ~~diagnose~~ identify ~~its~~ the genes therein.

[0003] ~~A~~ One purpose of the present invention is to provide a method for constructing a DNA library support, *i.e.*, ~~(original support)~~ an original support, on which A DNA library is immobilized ~~by utilizing~~ using a micro amount of the DNA library test material. Another purpose of the present invention is to provide a method for constructing the necessary number of replica supports. ~~Further~~Furthermore, another purpose of the present invention is to provide a support on which a replica ~~DNA piece~~ of DNA is immobilized.

~~DISCLOSURE OF THE INVENTION~~ SUMMARY OF THE INVENTION

[0004] ~~In a~~The method for constructing a cDNA (complementary DNA) library according to the present invention comprises, ~~the method is characterized of~~ hybridizing oligo

$(dT)_n$ on a support, and thereafter messenger ~~mRNA~~ RNA (~~messenger Ribosenucleic Acid,~~ hereinafter, ~~it is referred as "mRNA")~~, and ~~affecting~~ reacting it with ~~RT (Reverse Transcriptase~~reverse transcriptase ~~,~~ (hereinafter, ~~it is referred as "RT")~~ in order to immobilize complementary DNA.

[0005] In a method for constructing a cDNA library according to the present invention, mRNA is dehybridized from a cDNA library immobilized on a support. The method comprises ~~is characterized of~~ immobilizing the same cDNA library by ~~utilizing~~using ~~the~~ dehybridized mRNA.

[0006] In a method for constructing a ~~gDNA (~~genomic DNA~~)~~ (hereinafter, gDNA) library according to the present invention, ~~the method is characterized of ligasing~~ a double stranded gDNA is ligated to an oligo nucleotide on a support with restrictive enzyme portion.

[0007] ~~In a method for constructing~~ A single stranded gDNA library according to the present invention~~, the method is characterized of utilizing a sense portion of a~~ is produced by ligating double stranded gDNA with respect to an oligonucleotide on a support having a restrictive enzyme portion which ligates the double stranded gDNA. ~~immobilized on the support as recited in claim 3.~~

[0008] ~~In a~~ Another method for a single stranded gDNA library according to the present invention~~,~~ comprises ~~the method is characterized of~~ dehybridizing an anti-sense portion of the gDNA library ~~as recited in claim 3~~ obtained above, and ~~synthetic~~ synthetically immobilizing a sense portion ~~on~~of ~~a~~the gDNA on a support ~~by utilizing~~using the anti-sense portion.

[0009] In any ~~methods~~ of the processes according to the present invention ~~as recited in one of claims 1 to 5, it is characterized that a support is previously chemical modificated~~ the support has been previously chemically modified with a nucleotide or an oligo nucleotide.

[0010] ~~A~~ The substrate ~~according to~~of the present invention ~~is characterized in that~~ comprises a support on which a DNA library is immobilized by any one of the methods ~~as recited~~ described above. ~~i~~In ~~one of claims 1 to 6 . A support according to~~In another embodiment of the present invention, ~~is characterized in that~~ a single stranded DNA library is immobilized.

~~BRIEF DESCRIPTION OF THE DRAWINGS~~ BRIEF DESCRIPTION OF THE DRAWINGS

[0011] ~~Fig.~~ Figure 1 ~~shows~~is a schematic view of a device for constructing a cDNA library support according to the present invention.

[0012] ~~Fig.~~ Figure 2 ~~shows~~is a schematic view of a device for constructing a gDNA library according to the present invention.

[0013] ~~Fig.~~ Figure 3 ~~shows~~is a flow chart for explaining a process for constructing a cDNA library support according to the present invention.

[0014] ~~Fig.~~ Figure 4 ~~shows~~is a schematic view for explaining a process for constructing a cDNA library support according to the present invention.

[0015] ~~Fig.~~ Figure 5 is a flow chart for explaining a process of constructing a gDNA library support according to the present invention.

[0016] ~~Fig.~~ Figure 6 is a schematic view for showing a process for constructing a gDNA library support according to the present invention.

[0017] ~~Fig.~~ Figure 7 ~~shows~~ is an enlarged view of ~~a~~ support f.

[0018] ~~Fig.~~ Figure 8 ~~shows~~ is an enlarged view of ~~a~~ support e.

~~BEST MODE FOR CARRYING OUT~~

DETAILED DESCRIPTION OF THE INVENTION

[0019] ~~An~~ A chemically modified original support which has been ~~chemical modificated~~ chemically modified with respect ~~to~~ only to a nucleotide or oligonucleotide and a plurality of

supports for producing replicas ~~a replica use~~ are prepared so as to produce an original support ~~or~~ and replica supports. The supports are ~~set~~ introduced ~~in~~ into a replica constructing device.

[0020] A device for constructing a DNA library support according to the present invention will be explained with reference to ~~accompanying~~ the drawings. ~~Fig.~~ Figure 1 is a schematic view of a device for constructing a cDNA library support. ~~Fig.~~ Figure 2 is a schematic view of a device for constructing a ~~g~~cDNA library support. ~~Fig.~~ Figure 3 is a flow chart ~~for~~ explaining a process of constructing a cDNA library support. ~~Fig.~~ Figure 5 is a flow chart for explaining a gDNA library support.

[0021] ~~Fig.~~ Figure 1 ~~shows~~ is a schematic view of a device for automatically constructing and duplicating a cDNA library support. A device ~~A~~ for constructing a DNA library support as shown in ~~Fig.~~ Figure 1 comprises liquid feeding means 105 for feeding reaction solution to a container, a liquid feeding switch means 220 for stopping the flow of reaction solution and feeding new reaction solution, nozzle driving means 100 for driving a nozzle 101 for inleting/outleting test material in a front-rear and right-left direction in a plane and an upper-lower direction, solution temperature controlling means 30 for heating~~/~~ or cooling the reaction solution in the container, test material container holding means 20 for holding containers ~~in~~ into which each test material ~~and/~~or

solution ~~are~~is set for constructing ~~a~~ respective immobilized DNA library supports, and test material ~~container temperature~~ control means ~~40~~ for ~~controlling~~ maintaining the test material container holding means at a predetermined temperature, etc ~~and so on~~. It is preferable that the container holding means 10 ~~can~~ hold 96 ~~test material containers~~ or more ~~than~~ test material containers ~~in view of a~~to be ~~connection~~ connected to a PCR device and/or a PCR ~~product~~ analysis device ~~in near future~~for subsequent use. It is preferable that the test material ~~comprises~~ container holding means comprise at least four test material container inserting holes for replica supports. It is preferable that the number~~umber~~ of ~~the~~ container inserting holes provided at the test material container holding means 20 ~~is~~ be ten ~~10~~ holes or more, for subsequent~~than holes in view of a~~ connection to the PCR device and/or the PCR product analysis device. ~~in near future.~~ It is preferable that the container holding means 10 and the test material container holding means 20 ~~are~~ be an aluminum block with good conductivity ~~in view of~~for thermally controlling the solution temperature controlling means 30 and the test material container temperature control means 40.

[0022] It is preferable that a support for construction~~constructing~~ a DNA library support ~~is~~ be a plate shape, a ball shape, a cube shape or a grain shape in the both ~~cases of an~~the original support and replica supports. Although ~~a~~ the material of which the support is made is not

specified, it is preferable that the material ~~does~~ not react with reaction solution or that the material does not deposit harmful material when exposed~~with respect~~ to a DNA immobilization reaction. For example, plastic, glass, silicon, ~~silicone~~ and ~~metal~~ metals are preferred~~preferable~~ materials~~material~~. ~~The~~ A plate shape, ~~the~~ a ball shape, ~~the~~ a cube shape and ~~so on~~on the like are preferred~~preferable~~. Particularly, a support made of diamond or carbon atoms including diamond is preferred~~preferable~~.

Production of Original Support and its Replica Support with cDNA Library

~~(Production of Original support and its Replica support with cDNA Library)~~

[0023] Referring to Figures 1 and 3~~With reference to Fig. 1 and Fig. 3~~, ~~it will be explained~~ a process for constructing an original support and replica supports on which a cDNA library is immobilized and its ~~replica supports~~ will be explained. ~~At first~~ First, a necessary number (T1-Tn) of supports of 3 mm x 3 mm x 0.1 mm chemically modified~~modificated~~ with respect only to ~~oligo~~ (dT)-n, ~~(n is from 15 to 30)~~ are prepared before a DNA library is immobilized on these supports. ~~These~~ A supports ~~are chemically modificated~~ modified with ~~respect to only~~ oligo (dT)-n and ~~a DNA library has not been immobilized on the supports. A reason why a support chemically modified~~

~~with oligo (dT) n~~ is used ~~is~~ because it is easy to hybridize mRNA in ~~Total~~total RNA that has been chemically ~~modificated~~modified. These supports are inserted into containers CB1 to CBn and the containers are set ~~in~~into the container holding means 10. In such a case, it is preferable to insert one support into a first container in view of ~~certainly~~ constructing an original support as an immobilized cDNA library and its replica ~~supports~~support ~~by utilizing~~using a micro amount of mRNA obtained from a small amount of test material. Regarding an order of setting containers CB1-CBn into which a ~~support~~ chemically ~~modificated~~ modified support is inserted at the container holding means ~~10~~, the container CB1 ~~for~~ an original support in which a chemically modified ~~support T1 chemically modificated~~ is set is inserted into a first inserting portion HT1. The ~~Neccessary~~necessary number of containers (CB2-CBn) for replica supports into which the corresponding number of ~~supports~~ chemically ~~modificated~~ modified supports (T2-Tn) is set, respectively, are inserted into a second inserting hole HT2, … ~~a~~ to the n^{th} inserting hole HTn in order.

Production of Original Support

~~(Production of Original support)~~

[0024] Reaction solution 203, which includes~~including~~ purified total RNA solution 201, RT enzyme solution 202 and nucleotide (dT, dA, dG, dC) is set ~~in~~into the test material

container holding means 20 controlled at a predetermined temperature (*i.e.*, 4°C) ~~(i.e. 4 °C)~~. Tris ethylene diamine tetraacetic acid (hereinafter, TE) ~~Tris-Ethylene-diamine-tetraacetic-acid (hereinafter, it is referred as "TE")~~ solution 204 for cleaning/eluting DNA (buffer solution including TE) ~~buffer solution including Tris-Ethylene-diamine-tetraacetic acid)~~ and a waste solution tank 210 and others are provided. A capillary tube 230 is provided as a liquid feeding path by ~~connecting~~ connection to the liquid feeding switch means 220 for ~~liquid~~ feeding the respective solution. It is ~~preferable~~preferred that the capillary tube 230 ~~is an~~be a ~~anti~~-corrosion resistant stainless tube for liquid chromatography. It is ~~preferable~~ preferred that ~~a~~ the connection between the test material inlet nozzle 101 and the liquid feeding switch means 220 through the liquid feeding means 105 ~~is~~ be a silicone tube 231. Then~~,~~ the test material inlet nozzle 101 is moved to a position of the hole HT1 in the container holding means 10 so as to insert the nozzle 101 into the container CB1 in which the first support (T1) is set. The liquid feeding switch means 220 is provided at a side of the reaction solution, and the reaction solution is ~~inleted~~ fed to the container CB1 by driving the liquid feeding means 105. The liquid feeding switch means 220 is shifted to the total RNA solution 201 and the predetermined amount of the solution 201 is inleted by the liquid feeding means 105. After passing a predetermined time

(for example, 20 to 30 minutes) at a temperature equal to or lower than the predetermined temperature (for example 20°C), the liquid feeding switch means ~~220~~ is shifted to RT enzyme solution (enzyme 1) 202 so as to ~~inlet~~ introduce a predetermined amount of the RT solution 202 by driving the liquid feeding means 105. After ~~removing~~ the test material inlet nozzle 101 is removed from the container CB1, ~~a~~ the temperature of the container holding means 10 is set at the predetermined temperature (for example, 42°C), and the RT enzyme reaction for constructing cDNA from mRNA ~~is proceeded by maintaining~~proceeds for a predetermined time (for example 30 to 60 minutes). After ~~setting a~~the temperature of the container holding means 10 is set at ~~a~~ temperature equal to or lower than the predetermined temperature (for example, 20°C), the liquid feeding switch means 220 is shifted to the waste liquid tank 210 so as to discharge reaction solution in ~~the~~ container CB1 to the waste liquid tank 210 by driving the liquid feeding means 105. The liquid feeding switch means 220 is shifted to the TE solution 204 so as to ~~inlet~~ introduce a predetermined amount of the TE solution 204 into the container CB1 by driving the liquid feeding means 105. ~~A temperature of the~~The container holding means 10 is heated to a predetermined temperature (for example, 90°C) by driving the solution temperature control means 30, ~~so as to hybridize mRNA~~ at which temperature mRNA is hybridized. Then, the liquid feeding switch means 220 is shifted to ~~a~~ container 206 for ~~temporally~~

temporarily preserving mRNA, and dehybridized mRNA solution is moved to ~~the~~ container 206 for ~~temporally~~ temporarily preserving mRNA by driving the liquid feeding means 105.

~~(Production of Replica supports)~~**Production of Replica Supports**

[0025] ~~In the next~~Next, ~~it~~ will be described a method for constructing replica supports by re-using ~~mRNA dehybridized~~ dehybridized mRNA from the original cDNA library support produced by the above- described method. ~~At the first~~ First, after removing the test material inlet/outlet nozzle 101 from ~~the~~ container CB1, the nozzle 101 is moved to ~~a~~ container CB2 ~~in which~~containing a replica support (T2), ~~and mRNA solution 206 temporally~~ temporarily preserved, ~~is~~ inlet to the container CB2 by reversely driving the liquid feeding means 105. Then, steps ~~explained~~ described above for the production of the ~~above~~ original support are repeated. However, ~~a latter~~the step for ~~inleting~~introducing the Total RNA solution 201 can be omitted. The liquid feeding switch means 220 is provided at a side of the reaction solution 203 of ~~the~~ container CB2. The reaction solution is ~~inleted to the~~ introduced into container CB2 by driving the liquid feeding means 105. After ~~maintaining the~~ container CB2 has been maintained for a predetermined time (for example, 20 to 30 minutes) at a temperature equal to or lower than a predetermined temperature (for example, 20°C), the liquid

feeding switch means 220 is shifted to the RT enzyme solution (enzyme 1) 202 so as to ~~inlet~~ introduced a predetermined amount of the solution by driving the liquid feeding means 105. ~~After removing the~~The test material inlet/outlet nozzle is removed 101 from the container CB2, ~~a~~ and the temperature of the container holding means 10 is maintained at a predetermined temperature (for example, about 42°C) for a predetermined time (for example, 30 to 60 minutes). ~~After controlling~~The a temperature of the container holding means 10 ~~at~~ is set a temperature equal or lower than a room temperature (20°C), and the liquid feeding switch means 220 is shifted to the waste liquid tank 210. The test material inlet/outlet nozzle 101 is inserted into ~~the~~ container CB2, reaction solution in the container CB2 is discharged to the waste solution tank 210 by driving the liquid feeding means 105. The liquid feeding switch means 220 is shifted to the TE solution 204 so as to ~~inlet~~ introduce a predetermined amount of the TE solution 204 into ~~the~~ container CB2 by driving the liquid feeding means 105. Then, the liquid feeding switch means 220 is shifted to the waste liquid tank 210 so as to discharge the TE solution in ~~the~~ container CB2 into the waste liquid tank. By repeating the above process several times (~~equal or more than 5 times~~ preferably at least 5 times), a first replica support is produced which is a duplicate of the ~~duplicated from~~ the original cDNA library support ~~is produced~~. ~~Necessary~~ The necessary numbers of replica supports are

produced by repeating ~~a~~the cyclic operation for constructing the replica support ~~necessary~~the required number of times.~~Fig. 2 is a schematic view of a device automatically constructing and duplicating a gDNA library support.~~

[0026] The device for constructing a DNA library support as shown in ~~Fig.~~Figure 2 comprises a liquid feeding means 105 for liquid feeding reaction solution ~~and so on~~etc. to a container, a liquid feeding switch means 220 for switching the liquid feeding of the reaction solution, a nozzle, a nozzle driving means 100 for driving a test material inlet/outlet nozzle 101 in a front-rear direction and left-right direction in a plane and an upper-lower direction, a container holding means 10 for holding a container in which a support is set, a container solution temperature control means 30 for heating~~/~~ or cooling the reaction liquid in the container, a test material container holding means 20 for holding containers in which test materials and test solutions for duplicating an immobilized DNA library support are set, respectively, and a test material container temperature control means 40 for controlling the test material container holding means at a predetermined temperature. It is ~~preferable~~ preferred that at least 96 ~~test material containers or more than test~~ material test containers can be ~~insert to~~ inserted into the container holding means 10 in view of connecting to a PCR device and/or a PCR product analysis device ~~in near future~~ for subsequent operations. ~~It is preferable that~~Preferably, the test

material container holding means 20 ~~comprises~~ contains at least four holes for a test material container in order to produce replica supports. It is ~~preferable~~ preferred that ~~a~~ the number of the test material holes provided at the test material container holding means 20 ~~is equal or more than~~be at least 10 ~~in view of connecting~~for subsequent connection to a PCR device and/or a PCR product analysis device ~~in near future~~. It is ~~preferable~~ preferred that the container holding means 10 and the test material container holding means 20 ~~are~~ be made of aluminum with good thermal conductivity in ~~view of~~order to thermally ~~controlling the~~control container liquid temperature control means ~~30~~ 36 with Peltier element.

~~(Production of Original support of gDNA Library and Its Replica supports)~~ Production of Original Support of gDNA Library and its Replica Supports

[0027] Referring to Figures 2 and 5, the ~~With reference to Fig. 2 and Fig. 5, it will be explained a~~ production of an original support and replica supports on which immobilized ~~with~~ a gDNA library ~~and its replica supports~~will be explained. ~~Necessary~~ The necessary number (T1~Tn) of supports (for example, 3 mm x 3 mm x 0.1 mm) of supports chemically modified with oligonucleotide ~~(~~sense portion~~)~~ having a restrictive enzyme portion are prepared. With respect to the original support, ~~(~~T1~~)~~, oligo nucleotide (anti-sense portion) is

hybridized and treated with restrictive enzyme so as to prepare a complete restrictive enzyme portion. _The original support T1 is set in a container CB1. ~~and~~ Replica supports T2-Tn ~~(replica supports)~~are chemically ~~modificated~~ modified with ~~oligo nucleotide~~oligigonucleotide (sense portion) having restrictive enzyme portion are set in containers CB2-CBn. _ These containers are set in the solution holding means 10. _ ~~Regarding~~ With respect to a setting order, the container CB1 in which an original support T1 is set is inserted into a first inserting hole HT1 ~~at the first~~initially, and a second container and ~~the~~ successive containers CB2-CBn, in which each replica support is set, ~~are~~is inserted in order. _Reaction solution 303, including purified gDNA library solution 306 treated with restrictive enzyme, DNA ~~Ligase~~ ligase solution (enzyme 1) 305, DNA Polymerase solution (enzyme 2) 302 and nucleotide (dT, dA, dG, dC) 303 ~~is~~ are set ~~in~~ into a test material solution holding means 20 ~~of~~ in which ~~a~~ the temperature is ~~fixed~~ maintained at a predetermined temperature (24 °C). _TE solution for cleaning/eluting DNA 304 and a waste liquid tank 310 are provided. _A capillary tube 330 for the respective ~~solution~~ solutions is connected to a liquid feeding switch means 220. _It is ~~preferable~~ preferred that the capillary tube 230 ~~is an anti~~be a corrosion-resistant stainless tube for liquid chromatography. _

[0028] The liquid feeding switch means 220 and the test material inlet/outlet nozzle 101 and others are connected to ~~a~~ the front end of the capillary tube 230 through the liquid feeding means 105. A silicone tube 231 is ~~preferable~~ preferred for ~~its~~ this connection. A test material inlet/outlet nozzle 101 is moved to a location of the hole HT1 of the container holding means 10 so as to insert the nozzle 101 into the container CB1 in which the first support, ~~(T1)~~T1, is set. The liquid feeding switch means 220 is shifted to the reaction solution 303 so as to inlet a predetermined amount (for example, 17 μL) of the reaction solution 303 by driving the liquid feeding means 105. The liquid feeding switch means 220 is shifted to the gDNA library solution 306 treated with restrictive enzyme so as to ~~inlet~~ introduce a predetermined amount (for example, 2 μL) of the solution 306 by driving the liquid feeding means 105. After maintaining the container CB1 at a temperature equal to or lower than a predetermined temperature (for example, 20°C) for a predetermined time (for example, 20 to 30 minutes), the liquid feeding switch means 220 is shifted to DNA ~~Ligase~~ ligase solution (enzyme 1) 305 so as to ~~inlet~~ introduce a predetermined amount (for example, 1 μL) of the solution 305 into the container CB1 by driving the liquid feeding means 105. After removing the test material inlet/outlet nozzle 101 from ~~the~~ container CB1, ~~a~~ the temperature of the container holding means ~~10~~ is ~~controlled~~ maintained at a predetermined temperature (for example, 37°C).

After maintaining ~~the~~ container CB1 at this temperature for a predetermined time (for example, 30 to 60 minutes), the gDNA library immobilized with DNA ~~Ligase~~ ligase is produced on the support T1. After ~~controlling a~~ maintaining the temperature of the container holding means 10 at a predetermined temperature (for example, equal to or less than 20°C), the liquid feeding switch means 220 is shifted to the waste liquid tank 310 so as to insert the test material inlet/outlet nozzle 101 ~~to the~~ into container CB1 and discharge the reaction solution in ~~the~~ container CB1 by driving the liquid feeding means 105. The liquid feeding switch 220 is shifted to the TE solution 304, a predetermined amount (for example, 500 μL) of the TE solution is ~~inleted to the~~introduced into container CB1 by driving the liquid feeding means 105. Then, the liquid feeding switch means 220 is shifted to the waste liquid tank 310 so as to discharge the TE solution in the container CB1. By repeating the process several times (for example, ~~equal or more than 5~~ at least five times), the immobilized support T1 is cleaned. After ~~cleaning~~ the immobilized support T1 has been cleaned, the liquid feeding switch means 220 is shifted to the reaction solution 303 so as to ~~inlet~~ introduce a predetermined amount (for example, 19 μL) of the reaction solution 303 into ~~the~~ container CB1 by driving the liquid feeding means 105. By ~~heating a~~ maintaining the temperature of the container holding means 10 at a predetermined temperature (for example, 90°C), and maintaining the container

CB1 at this temperature for a predetermined time (for example, 10 to 20 minutes), anti-sense portion is dehybridized from the immobilized gDNA library. Then, the liquid feeding switch means 220 is shifted to ~~the~~ container 306 in which a gDNA library (anti-sense portion) is ~~temporally~~ temporarily preserved so as to ~~outlet~~ remove the gDNA library (anti-sense portion) solution from ~~the~~ container CB1. ~~In the present stage, a production of~~ This produces the first gDNA library (sense portion) support (original support) ~~is accomplished~~.

~~(Production of Replica support)~~ **Production of Replica Supports**

[0029] ~~After removing the~~ The test material inlet/outlet nozzle 101 is removed from ~~the~~ container CB1~~, the nozzle 101 is~~ and moved to the next container, CB2, in which a replica support (T2) is set so as to ~~inlet~~ introduce the gDNA library (anti-sense portion) solution 306 ~~temporally~~ temporarily preserved into ~~the~~ container CB2. In order to produce a replica support, the above-described cyclic operation is repeated as necessary ~~times~~ so as to produce ~~necessary~~ the required number of replica supports. However, in the second embodiment described below, it is noted that DNA ~~Polymerase~~ polymerase solution (enzyme 2) 302 is selected during a replica constructing process so as to ~~inlet~~ introduce a predetermined amount (for example, 1 μL) of ~~the~~ solution 302 into ~~the~~ container CB2 by driving the liquid feeding means 105.

~~Embodiments~~ **Embodiments**

~~(Embodiment 1)~~ **Embodiment 1**

[0030] ~~With reference to Fig. 1, Fig. 3 and Fig. 4, it will be explained a~~ This embodiment, referring to Figures 1, 3, and 4, explains production of an original support immobilized ~~with~~ a cDNA library, and ~~its~~ production of replica supports. ~~Regarding pre-treatment of the test material, the~~ The test material is prepared by (1) breaking ~~cell~~ cells and ~~tissue~~ tissues and purifying total RNA (see ~~a step~~ Step S1 in ~~Fig.~~Figure 3). ~~Regarding a pre-treatment of~~ For pre-treating a support immobilized with a cDNA library, ~~rat's~~ a piece of rat liver tissue of about 5 mm x 5 mm is homogenized in a test material kit (for example, ISOGEN sold by K.K. Nippon Gene) and the total RNA is purified in accordance with its protocol. Ten supports (T1~T10) of 3 mm x 3 mm x 0.1 mm are chemically ~~modificated~~ modified with oligo (dT)n (wherein n is from 15 to 30), ~~are prepared (see the step S2 in Fig. 3)~~as shown in Step 2 in Figure 3. The supports ~~(~~T1-T10~~)~~, immobilized with an amino group on ~~its~~ their ~~surface~~ surfaces, are treated with activating dihydric carbonic solution. After ~~cleaning~~ being cleaned with ethanol and distilled water in that order, the supports are maintained in the oligo (dT)n solution for ~~10~~ ten minuets. Each support ~~(T1-T10)~~ is inserted into ~~a~~ respective containers CB1~CB10, individually~~,~~. ~~the~~ The containers ~~CB1-CB10~~ are set ~~in~~ into the temperature controlling aluminum block 10. Reaction solution 203 including purified total RNA

solution 201, RT enzyme solution 202 and nucleotide (dT, dA, dG, dC) are set ~~in~~ into a low temperature test material aluminum block 20 ~~controlled~~ in which the temperature is maintained at 4°C. TE solution (buffer liquid including ~~Tris-Ethylene-diamine-tetraacetic-acid~~ tris-ethylene diamine tetraacetic acid) for cleaning DNA and the waste liquid tank 210 are provided at an exterior side of the low temperature test material aluminum block 20. The total RNA solution 201, the RT enzyme solution 202, the reaction solution 203, the TE solution 204, and the waste liquid tank 210 ~~is~~ are connected to an automatic 8-~~ways~~ way switching valve 220 through capillary tubes 230, respectively. A test material inlet/outlet capillary needle 101 is moved to ~~a~~ the location of the inlet hole HT1 of the temperature control container aluminum block 10 so as to insert the capillary needle 101 into ~~the~~ container CB1 in which the first support T1 (original support) is set. The automatic 8-~~ways~~ way switching valve 220 is shifted to ~~the~~ reaction solution 203 so as to ~~inlet the reaction solution 203 of~~introduce 17 μL of the reaction solution into ~~the~~ container CB1 by driving a peristaltic pump 105. The automatic 8-~~ways~~ way switching valve 220 is shifted to the ~~Total~~ total RNA solution 201, and 2μL of ~~the~~ solution 201 ~~of 2 μL~~ is pumped by the peristaltic pump 105. In order to hybridize oligo (dT) immobilized on a surface of the support and mRNA in the ~~Total~~ total RNA solution, the solutions are maintained at 20°C for 20 minutes (see ~~a~~ step S3

in ~~Fig.~~Figures 3 and ~~Fig. 4(a)~~). After ~~passing the~~this time, the automatic 8-~~ways~~ ways switching valve 220 is shifted to ~~the~~ RT enzyme solution 202 ~~of~~ 1 μL ~~so as to pump~~ of the RT enzyme solution 202 by means of the peristaltic pump 105. After ~~removing~~ the test material inlet/outlet capillary needle 101 is removed from the container CB1, the container holding means 10 is ~~controlled~~ maintained at 42°C for 30 minutes so as to produce a cDNA library immobilized on ~~the~~ support T1 ~~(immobilized support~~) by the RT enzyme (see a step S4 in ~~Fig.~~Figures 3 and ~~Fig.~~ 4 ~~(b)~~). After ~~cooling~~ the container holding means is cooled ~~10~~ to 10-20°C ~~again~~, the automatic 8-~~ways~~ways switching valve 220 is shifted to the waste liquid tank 210 ~~so as~~ to insert the test material inlet/outlet capillary needle 101 into ~~the~~ container CB1. The reaction solution in ~~the~~ container CB1 is discharged to the waste liquid tank 210 by driving the peristaltic pump 105. The automatic 8-way switching valve 220 is shifted to the TE solution 204. ~~The TE solution 204 of 500~~Five hundred μL of TE solution 204 is ~~inleted to the~~introduced into container CB1 by driving the peristaltic pump 105. Then the automatic 8-~~ways~~ way switching valve 220 is shifted to the waste liquid tank 210 so as to discharge the TE solution in the container CB1 to the waste solution tank 210. By repeating ~~the~~ this operation several times (at least five ~~time or more than~~ times), ~~the~~ immobilized support T1 is cleaned (see ~~a~~ step S5 in ~~Fig.~~Figure 3). After ~~cleaning the~~ immobilized support T1 is cleaned, the

automatic 8-~~ways~~ way switching valve 220 is shifted to the reaction solution 203, ~~the reaction solution 203 of~~ 19 μL of reaction 203 is ~~inleted to the~~ introduced into container CB1 by ~~proving~~ activating the peristaltic pump 105. ~~The container~~Container holding means 10 is heated to 90°C, mRNA is dehybridized from the immobilized cDNA library after ~~maintaining~~ holding the support at this temperature for ~~10~~ ten minutes (see ~~a~~ step S6 in ~~Fig.~~Figures 3 and Fig. 4 ~~(~~d~~)~~). ~~In the next~~Next, the automatic 8-~~ways~~ way ~~switch~~ switching valve 220 is shifted to ~~the~~ container 206 in which mRNA is ~~temporally~~ temporarily preserved, dehybridized mRNA solution is eluted and ~~outleted~~ removed from ~~the~~ container CB1 and temporarily preserved in ~~the~~ container 206 ~~temporally~~ (see a step S7 in ~~Fig.~~Figure 3). In accordance with the above steps, the first cDNA library support, ~~(~~the original support~~)~~ on which ~~immobilized with~~ a cDNA library is immobilized, is produced (see ~~a~~ step S8 in ~~Fig.~~Figures 3 and ~~Fig.~~ 4 ~~(~~c~~)~~). After ~~removing~~ the test material inlet/outlet capillary needle 101 has been removed from ~~the~~ container CB1, ~~the~~ capillary needle 101 is moved to ~~the~~ container CB2 in which a replica support, ~~(~~T2~~)~~, is set. ~~The replica~~Replica support (T2) ~~is~~ has been previously chemically ~~modificated~~modified. The automatic 8-~~ways~~ way switching valve 220 is shifted to ~~the~~ container 206 for ~~temporally~~ temporarily preserving mRNA, ~~mRNA of~~and 19 μL of ~~temporary~~ temporarily preserved mRNA is ~~inleted~~introduced to ~~the~~ container CB2 by driving ~~a~~ peristaltic pump ~~105~~ 104

(see ~~an~~ arrow R as shown in ~~Fig.~~Figures 4~~(d)~~ to ~~Fig. 4(a)~~). In order to hybridize immobilized oligo (dT) and mRNA, ~~the~~ container CB2 is maintained at 20°C for 20 minutes. The automatic 8-~~ways~~ way switching valve 220 is shifted to the RT enzyme solution 202~~,~~ and ~~the RT enzyme solution 202 of~~ 1 μL of RT enzyme solution 202 is ~~inleted to the~~introduce into container CB2 by driving the peristaltic pump 105 (see ~~a~~ step S9 in ~~Fig.~~Figure 3). After ~~removing~~ the test material inlet/outlet capillary needle 101 has been removed from ~~the~~ container CB2, the temperature of the container holding means 10 is controlled at 42°C for 30 minutes so as to produce a cDNA library support immobilized on ~~the~~ support ~~(~~T2~~)~~ by RT enzyme (see a step S10 in ~~Fig.~~Figures 3 and ~~Fig.~~ 4 ~~(b)~~). After ~~cooling~~ the container holding means is cooled to 10 to 20°C ~~again~~, the automatic 8-~~ways~~way switching valve 220 is shifted to the waste liquid tank 210. The test material inlet/outlet capillary needle 101 is inserted into ~~the~~ container CB2 so as to discharge the reaction solution in ~~the~~ container CB2 to the waste liquid tank 210 by driving the peristaltic pump 105. The automatic 8-~~ways~~ way switching valve 220 is shifted to the TE solution 204, ~~the TE solution 204 of~~ 500 μL of TE solution 204 is ~~inleted~~introduced into ~~the~~ container CB2 by driving the peristaltic pump 105. Then, the automatic 8-~~ways~~ way switching valve 220 is shifted to the waste liquid tank 210 so as to discharge the TE solution in ~~the~~ container CB2. By repeating the above operation ~~5~~ five

times, ~~the~~ immobilized support T2 is cleaned (see ~~a~~ step S11 in ~~Fig.~~Figure 3). After ~~cleaning the~~ immobilized support T2 is cleaned, the automatic 8-~~ways~~ way switching valve 220 is shifted to ~~the~~ reaction solution 203~~,~~. ~~the reaction solution 203 of~~Then 19 μL of reaction 203 is ~~inleted to the~~introduced into container CB2 by driving the peristaltic pump 105. In the next step, the container holding means 10 is heated~~,~~ to 90°C and maintained at that temperature for 10 minutes so as to dehybridize mRNA from the immobilized cDNA library (see ~~a~~ step S12 in ~~Fig.~~Figure 3). In the next step, the automatic 8-~~ways~~way switching valve 220 is shifted to the container 206 for ~~temporally~~ temporarily preserving mRNA, dehybridized mRNA solution is separated~~,~~ and eluted from ~~the~~ container CB2 by driving the peristaltic pump ~~5~~105 (see ~~Fig.~~Figure 4 ~~(d)~~) and ~~temporally~~temporarily preserved in ~~the~~ container 206 (see ~~a~~ step S13 in ~~Fig.~~Figure 3). In accordance with the above steps, a replica cDNA library support (replica support) is produced (see ~~a~~ step S14 in ~~Fig.~~Figures 3 and ~~Fig. 4 (c)~~.

[0031] With respect to the containers CB3-CB10 in which supports T3-T10 ~~is set~~are inserted, respectively, ~~the~~ a similar process is ~~operated~~conducted. By repeating the above steps S9 through S14 in order, eight replica supports are produced ~~in order~~. By utilizing supports ~~(~~T1-T10~~)~~ immobilized with a cDNA library of ~~rat's~~ rat liver tissue, a gene is amplified by a PCR device with respect to 18S rRNA. ~~It is confirmed the cDNA library is immobilized by an~~ AN

electrophoresis device is used to confirm that the cDNA Library is immobilized. ~~As the result~~Thus, it can be confirmed that the original support T1 and the replica supports T2-T10 are produced as normal cDNA library supports.

~~(Embodiment 2)~~**Embodiment 2**

[0032] ~~With reference to Fig. 2, Fig. 5, Fig. 6 and Fig. 7, it will be explained a production~~ Production of an original support on which is immobilized with a gDNA library and its replica supports are explained with reference to Figures 2, 5,6, and 7. ~~Regarding the pre-treatment of the test material, test~~ Test materials are prepared by (1) breaking ~~cell~~ cells and ~~tissue~~ tissues and (2) purifying and treating gDNA with restrictive enzyme (see ~~a~~ step S21 in ~~Fig.~~Figure 5). Ten supports chemically ~~modificated~~ modified with a sense portion of oligo nucleotide a having base sequence of a target restrictive enzyme portion are prepared. ~~A~~ The size of the support is about 3 mm x 3 mm x 0.1 mm (see ~~a~~ step S22 in ~~Fig.~~Figure 5). A concept of the support is shown as ~~a~~ support f surrounded with a ~~breaking~~ broken line in ~~Fig.~~Figure 6~~(d)~~. ~~Fig.~~Figure 7 is an enlarged view of the portion. By ~~utilizing~~ using one ~~support~~ chemically ~~modificated~~modified support, an anti-sense portion of the oligo nucleotide is hybridized. The support is treated with restrictive enzyme so as to produce one support, ~~(T1)~~, having a complete restrictive enzyme cut portion (see ~~a~~ step S23 in ~~Fig.~~Figure 5 and an arrow (1) in

~~Fig.~~Figure 6 ~~(d)~~ to ~~Fig. 6 (a)~~). A concept of the support is shown as a support e surrounded with a broken line in ~~Fig.~~Figure 6 ~~(a)~~. ~~Fig.~~Figure 8 is an enlarged view of ~~its~~ this portion.

[0033] ~~A container CB1 in which the support~~Support TI is inserted into container CB1, and nine supports chemically ~~modificated~~ modified with a sense portion of oligonucleotide having the restrictive enzyme portion are ~~set in~~introduced into the container holding means 10. As shown in ~~Fig.~~Figure 2, the reaction solution 303, including purified gDNA library solution 306 treated with restrictive enzyme, DNA solution (enzyme 1) 305, DNA Polymerase solution (enzyme 2) 302 and nucleotide (dT, dA, dG, dC) ~~is~~ are set ~~in~~ into a low temperature test material aluminum block 20 in which the temperature is controlled at 4°C. TE solution 304 for cleaning/eluting DNA and a waste liquid tank 310 are provided at an exterior side of the low temperature test material aluminum block 20. As shown in ~~Fig.~~Figure 2, ~~the~~ reaction solution 303 including the gDNA (genomic DNA) library solution 306, the DNA Ligase solution (enzyme 1) 305, the DNA ~~Polymerase~~ polymerase solution (enzyme solution 2) 302 and nucleotide (dT, dA, dG, dC), ~~the~~ TE solution 304 for cleaning/eluting DNA and the waste liquid 310 are connected to an automatic 8-~~ways~~ way switching valve 220 through a capillary tube 230, respectively. The test material inlet/outlet capillary needle 101 is moved to a location of

the hole HT1 of the temperature control container aluminum block 10 so as to insert the capillary needle 101 into the container ~~CBB~~ CB1 in which the first support T1 (original support) is set. The automatic 8-~~ways~~ way switching valve 220 is shifted to the reaction solution 303, and ~~the reaction solution 303 of~~ 17 μL of reaction solution 303 is ~~inleted to the~~introduce into container CB1 by driving the peristaltic pump 105. The automatic 8-~~ways~~ way switching valve 220 is shifted to the gDNA library solution 306 treated with restrictive enzyme, ~~the solution 306 of~~ 2 μL of this solution is pumped by the peristaltic pump. After ~~maintaining~~ the has been maintained at 20°C for 20 minutes, the automatic 8-~~ways~~ way switching valve 220 is shifted to DNA ~~Ligase~~ ligase solution (enzyme 1) 305 so as to ~~inlet the solution 305 of~~introduce 1 μL of solution 305 to the container CB1 by driving the peristaltic pump 105. After removing the test material inlet/outlet capillary needle 101 from the container CBI, ~~a~~ the temperature of the container holding means 10 is ~~controlled~~ maintained at 37°C for 30 minutes so as to produce the gDNA library immobilized by DNA ~~Ligase~~ ligase on the support T1 (see ~~a~~ step S25 in ~~Fig.~~Figure 5 and an arrow 2 in ~~Fig.~~Figures 6 ~~(a) to Fig.~~and 6 ~~(b)~~). After ~~cooling~~ the container holding means is cooled to 10 to 20°C, the automatic 8-~~ways~~ way switching valve 220 is shifted to the waste liquid tank 310 so as to discharge the reaction solution in the CB1 by driving the peristaltic pump 105. The automatic 8-~~ways~~ way

switching valve 220 is shifted to the TE solution 304~~, the TE solution of~~ and 500 μL of TE solution is ~~inleted to the~~introduced into container CB1 by driving the peristaltic pump 105. ~~Then, the automatic 8-ways switching valve 220 is shifted to the waste liquid tank 310, the TE solution in the container CB1 is discharged.~~ By repeating these steps five times or more, ~~the~~ support T1 is cleaned (see ~~a~~ step S26 in ~~Fig.~~Figure 5). After cleansing the support T1, the automatic 8-~~ways~~ way switching valve 220 is shifted to the reaction solution 303. ~~The reaction solution of 19~~Nineteen μL of reaction solution is ~~inleted to~~introduced into ~~the~~ container CB1 by driving the peristaltic pump 105. The temperature control container aluminum block 10 is heated to 90°C and maintained for 10 minutes, an anti-sense portion is dehybridized from a double stranded ~~of a~~ sense portion and the anti-sense portion of the immobilized DNA library (see ~~a~~ step S27 in ~~Fig.~~Figure 5 and ~~an~~ arrow (3) ~~(M~~ in ~~Fig.~~Figures 6~~(b)~~ and ~~Fig.~~ 6~~(c)~~). The automatic 8-~~ways~~ way switching valve 220 is shifted to a container 306 for ~~preserving temporally~~temporary preservation so as to elute the anti-sense portion of the gDNA library solution from ~~the~~ container CB1 by driving the peristaltic pump 105 (see ~~a~~ step S29 in ~~Fig.~~Figure 5 and ~~an~~ arrow in ~~Fig.~~Figures 6~~(b)~~ ~~to Fig.~~ and 6 ~~(d)~~). ~~On the other hand, the~~The sense portion is only immobilized on ~~the~~ support ~~(T1)~~. Thus, the first support, that is, single stranded gDNA library support TI, ~~(~~original support~~)~~, is

produced (see ~~a~~ step S28 in ~~Fig.~~Figures 5 and ~~Fig.~~ 6 ~~(c)~~. After removing the test material inlet/outlet capillary needle 101 from the container CB1, the capillary needle 101 is moved to ~~the~~ container CB2 ~~in~~ which holds the support T2~~ is set~~. The reaction solution including nucleotide is added to ~~the~~ container CB2, ~~preserved~~which is maintained at 20°C. The gDNA library solution 306 including only anti-sense portion ~~temporally~~ temporarily preserved is ~~inleted to the~~introduced into container CB2 and maintained for 20 minutes (see ~~a~~ step S30 in ~~Fig.~~Figure 5). In the next step, DNA ~~Polymerase~~ polymerase is added, container CB2 is heated to 37°C and maintained at his temperature for one hour. As ~~the~~ a result, a double stranded gDNA library, of which a sense portion is immobilized on the support T2, is produced (see ~~a~~ step S31 and ~~an~~ arrow (4) in ~~Fig.~~Figures 6 ~~(d) to Fig.~~and 6 ~~(b)~~). ~~The container~~Container CB2 in which the support T2 is immobilized with the above double stranded gDNA library is~~ set is controlled~~ maintained at 20°C. After shifting the automatic 8-~~ways~~ way switching valve 220 to the waste liquid tank 310, the test material inlet/outlet ~~the~~ capillary needle 101 is ~~inlet~~ introduced into ~~the~~ container CB2. The reaction solution in ~~the~~ container CB2 is discharged by driving the peristaltic pump 105. The automatic 8-~~ways~~ way switching valve 220 is then shifted to the ~~TE solution 304, the TE solution of 500 g L is inleted to the container CB2 by driving the peristaltic pump 105. Then, the automatic 8-ways switching~~

~~valve 220 is shifted to the~~ waste liquid tank 310, and the TE solution in the container CB2 is discharged. By repeating the above steps ~~5 times or more than~~at least five times, ~~the~~ support T2 is cleaned (see a step S32 in ~~Fig.~~Figure 5). After ~~cleaning the~~ support T2 has been cleaned, the automatic 8-~~ways~~ way switching valve 220 is shifted to ~~the~~ reaction solution to 303 ~~so as to inlet the reaction solution of~~introduce 19 μL of this reaction solution into ~~the~~ container CB2 by driving the peristaltic pump 105. The aluminum block 10 is heated to 90°C and maintained at that temperature for ~~10~~ten minutes ~~so as to~~ dehybridize the anti-sense portion from immobilized gDNA library with double stranded ~~of the~~ sense portion and the anti-sense portion (see ~~a~~ step S33 in ~~Fig.~~Figure 5 and ~~an~~ arrow (5) in ~~Fig.~~Figure 6 ~~(b)~~ ~~and~~to ~~Fig.~~ 6 ~~(c)~~). The automatic 8-~~ways~~ way switching valve 220 is shifted to a container for ~~preserving temporary~~temporarily preservation so as to outlet the anti-sense portion of gDNA library solution from the container CB2 (see ~~a~~ step S35 in ~~Fig.~~Figure 5 and an arrow in ~~Fig.~~Figure 6 ~~(b)~~ to ~~Fig.~~ 6 ~~(d)~~. A second single stranded gDNA library support, that is, a replica support T2 on which a sense portion is immobilized, is produced (see ~~a~~ step S34 in ~~Fig.~~Figures 5 and ~~Fig .~~ 6 ~~(c)~~). By repeating the above steps, a double stranded gDNA library is immobilized on a support. An anti-sense portion is dehybridized from the double stranded immobilized gDNA library so as to produce ~~remained~~ the remaining number (T3-T10) of single stranded gDNA

library supports (replica supports). That is, ~~a~~ the process including steps as shown in ~~Fig.~~ Figures 6 ~~(b), 6 (d), Fig. 6 (b)~~ and ~~Fig.~~ 6 ~~(d)~~ is repeated so that any number of supports on which the same single stranded gDNA library is immobilized can be produced ~~(see Fig. 6 (e))~~.

~~POSSIBILITY OF USE IN THE INVENTION~~

[0034] ~~In a device according to the~~The present invention~~, a production of~~ produces a support immobilized with cDNA library from mRNA and a gDNA library treated with a restrictive enzyme of gDNA ~~can be produced easily~~. Although it is impossible to produce replica supports duplicated from DNA library solution in ~~a~~the conventional art, ~~necessary~~the required number (until mRNA and gDNA are chemically or physically ~~broken~~denatured) of replica supports ~~are easily produced as immobilized DNA supports for a short time~~can be produced by the method of the present invention. An immobilized DNA library support and its replica supports can be produced by collecting micro ~~amount~~ amounts of gene material from cultured ~~cell~~cells or ~~tissue~~tissues of an important ~~detected~~ object ~~at one time~~to be detected. ~~With respect to the same kind of test materials, various~~ Various kinds of gene research and detection can be ~~operated~~effected using the same kinds of test materials. ~~It is unaccountable benefit.~~ By ~~utilizing~~using the immobilized DNA library support and its replica supports, ~~budget and manual work for developing a new gene diagnosis technology would be remarkably saved in future~~developing a new gene diagnosis

technology can be more cheaply and easily than by using conventional techniques. If blood is collected from a patient or tissue is collected in ~~his~~ a medical ~~operation~~ procedure at one time for gene ~~diagnosing~~diagnosis, ~~re-use of the collected blood/tissue can be easily accomplished with respect to preventive medical research~~the collected blood or tissue can be easily re-used with respect to preventive medical research~~.~~, ~~Since~~ since a plurality of immobilized DNA library supports ~~are produced semi-eternally~~can be produced over a long period of time. ~~These facts can bring a big benefit by~~The process of the present invention makes it possible to perform many diagnoses on a patient from sample, reducing mental and/or economic ~~damage with respect to a patient as less as possible~~stress on a patient.

~~**[Name of the document]** ABSTRACT~~

~~A purpose of the present invention relate to provide a method for constructing an original support on which a DNA (Deoxyribonucleic Acid) library and others are immobilized by utilizing micro amount of DNA test material, a method for constructing its replica supports and a support on which a duplicated DNA piece is immobilized in a molecular biological technology and/or a biochemical technology such as a gene technology, a protein technology, a cell technology and immunology. In a method for constructing a cDNA (complemetary DNA) library according to the present invention, Reverse Transcriptase enzyme is affected so as to immobilize the complementary DNA on a support after hybridizing mRNA (messenger RNA). Alternatively, mRNA is dehybridized from a cDNA library immobilized on a support and then the same cDNA library is immobilized on a support by utilizing the mRNA. In a method for constructing a genomic DNA (gDNA) library, a double stranded gDNA is ligased, the gDNA library is immobilized on a support. Alternatively, after the ligation of the double stranded gDNA, a sense portion of the gDNA is immobilized on a support. After dehybridizing an anti-sense portion of the gDNA library, the anti-sense portion is immobilized on a support.~~